Resistance Mutations to Zidovudine and Saquinavir in Patients Receiving Zidovudine plus Saquinavir or Zidovudine and Zalcitabine plus Saquinavir in AIDS Clinical Trials Group 229

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The relationships among treatment regimens, plasma human immunodeficiency virus (HIV) RNA levels, and resistance mutations to saquinavir (codons 48 and 90) and zidovudine (codon 215) were examined in a cohort of 144 patients from the AIDS Clinical Trials Group 229 study. After 24–40 weeks of therapy, no patients who had received the two-drug combination (zidovudine plus saquinavir) had only codon 48 mutations, 45.8% had only codon 90 mutations, and 8.3% had both codon 48 and 90 mutations. Mutations developed by patients who had received the three-drug combination (zidovudine and zalcitabine plus saquinavir) were codon 48 alone in 1.4%, codon 90 alone in 33.3%, and both codons 48 and 90 in 4.2%. The difference between the groups showed a trend toward reduced mutations with three versus two drugs but did not reach significance ($P = .11$, two-sided χ^2). Higher baseline HIV RNA levels correlated with the development of protease mutations. Mutations at codon 215 were present in 82% of all patients at baseline and in 87% after therapy.

Combination therapy is currently the standard of care in human immunodeficiency virus (HIV)–infected patients [1, 2]. Due to the highly effective nature of potent antiretroviral drug regimens, insight into the development of resistance mutations and loss of plasma HIV RNA suppression may require large, expensive studies with prolonged periods of follow-up. Study of earlier clinical trials performed with less potent combinations of drugs and in drug-experienced patients, in whom drug resistance and failure developed more rapidly than with antiretroviral-naive patients, may provide insight into some of the causes leading to combination therapy failure [3].

The importance of suppressing plasma HIV RNA with combination therapy to obtain a more durable response has emerged from recent studies [4, 5]. This suppression reflects reduced viral replication, thus minimizing the opportunities for HIV to develop drug resistance mutations [5, 6]. Although the correlation between virus load reduction and duration of response and improved prognosis has been well documented in large studies [5, 7], fewer data are available about these features and protease drug resistance mutations.

Combinations including a greater number of antiretroviral agents are now commonly prescribed due to their clinical success [8–10]. Although three- and four-drug combinations are being studied and used [10, 11], the degree to which the addition of a third agent to a double drug combination actually reduces the rate at which resistance mutations develop has not been well studied.

We sought to gain insight into these questions by examining the relationships among treatment regimens, plasma HIV RNA virus load levels, and the presence of resistance mutations to saquinavir and zidovudine in a large cohort of patients from the AIDS Clinical Trials Group (ACTG) 229 study in which zidovudine-experienced patients were randomized to receive zidovudine plus zalcitabine, zidovudine plus saquinavir, or zidovudine and zalcitabine plus saquinavir.

Methods

Patients. ACTG 229 was a double-blind randomized study that enrolled 302 patients with a baseline CD4 T cell count of 50–300/μL and at least 6 months of prior zidovudine therapy to receive one of three treatments, zidovudine plus zalcitabine, zidovudine plus saquinavir (hard-gel capsule), or zidovudine and zalcitabine plus saquinavir (hard-gel capsule). Patients had a median of 27 months of prior zidovudine therapy. Of the 302 enrolled, 285 completed the initial 24 weeks of study [3]. We examined all available stored samples from the zidovudine plus saquinavir and zidovudine and zalcitabine plus saquinavir arms of the study for resistance mutations to saquinavir and zidovudine. Patients from the non–saquinavir-containing zidovudine plus zalcitabine arm were not studied, as they were not expected to develop saquinavir resistance mutations.

Mutations. Mutations to saquinavir at codons 48 (G to V) and 90 (L to M) in the HIV-1 protease gene were determined from frozen plasma from samples taken at baseline and after 24–40 weeks of therapy. The presence of a wild type (WT) or mutant

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Table 1. Mutations at codons 48 and 90 in patients receiving either zidovudine (ZDV) plus saquinavir (SAQ) or ZDV and zalcitabine (ddC) plus SAQ.

	Baseline			⩾24 weeks		
	Codon 48	Codon 90	Codons 48 and 90	Codon 48	Codon 90	Codons 48 and 90
ZDV + SAQ	0% (0/60)	0% (0/60)	0% (0/60)	0% (0/72)	45.8% (33/72)	8.3% (6/72)
ZDV + ddC + SAQ	0% (0/57)	0% (0/57)	0% (0/57)	1.4% (1/72)	33.3% (24/72)	4.2% (3/72)
All patients	0% (0/117)	0% (0/117)	0% (0/117)	0.7% (1/144)	39.6% (57/144)	6.3% (9/144)

(MU) genotype was determined using a selective polymerase chain reaction method as previously described [12]. In addition, the presence of the codon 215 mutation to zidovudine was determined by a similar technique [13].

Statistical analysis. Plasma HIV RNA levels and CD4 T cell counts had been previously determined for each patient as part of the ACTG 229 clinical trial [3]. These data were obtained from the ACTG 229 database and were correlated with the presence of WT or MU genotype for the resistance mutations studied.

Results

Samples from 144 of the 149 patients who remained on therapy were analyzed for mutations at codons 48 and 90 of the protease gene after 24–40 weeks of treatment. Of these patients, 135 were at week 24, 7 at week 32, and 2 at week 40. Seventy-two patients had received treatment with zidovudine plus saquinavir and an equal number had received zidovudine and zalcitabine plus saquinavir. In addition, for 60 patients in the zidovudine plus saquinavir and for 57 in the zidovudine and zalcitabine plus saquinavir groups, baseline samples taken before therapy started were also analyzed. All baseline samples in both treatment groups were WT for codons 48 and 90 (table 1).

Following therapy with the two-drug combination in 72 patients, none had developed the codon 48 mutation alone, 45.8% (33) had developed the codon 90 mutation alone, and 8.3% (6) had developed both codons 48 and 90 mutations. Of the 72 patients receiving the triple combination, 1.4% (1 patient) had developed the codon 48 mutation alone, 33.3% (24) had developed the codon 90 mutation alone, and 4.2% (3) had developed both codon 48 and 90 mutations. The rate of mutations developing after treatment for both groups ($n = 144$) combined was 0.7% (1 patient), 39.6% (57), and 6.3% (9) for codon 48 alone, 90 alone, and 48 and 90 together, respectively. The de-

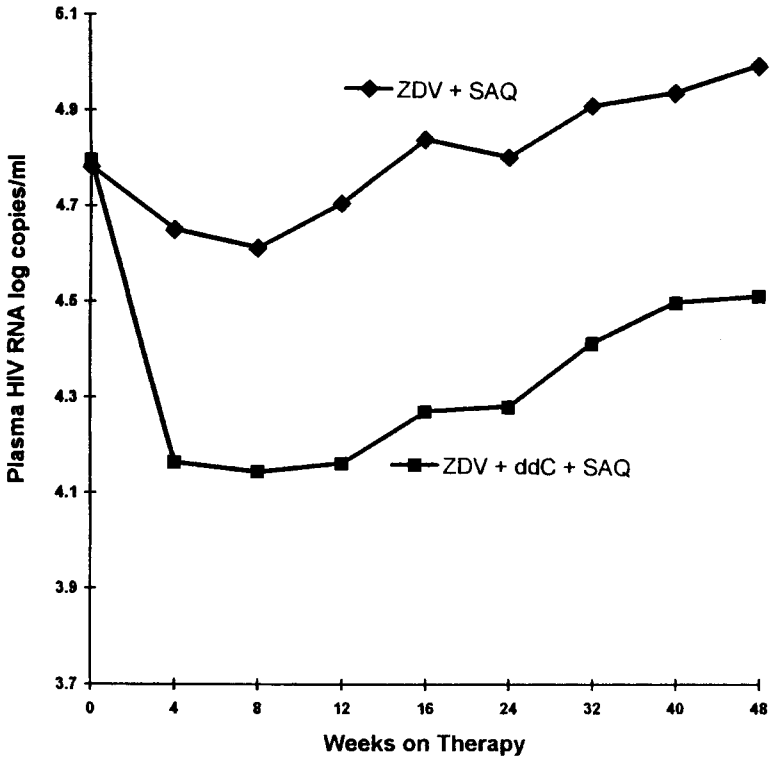

Figure 1. Changes in plasma human immunodeficiency virus RNA levels in patients receiving therapy with either zidovudine plus saquinavir (ZDV + SAQ) or zidovudine and zalcitabine plus saquinavir (ZDV + ddC + SAQ).

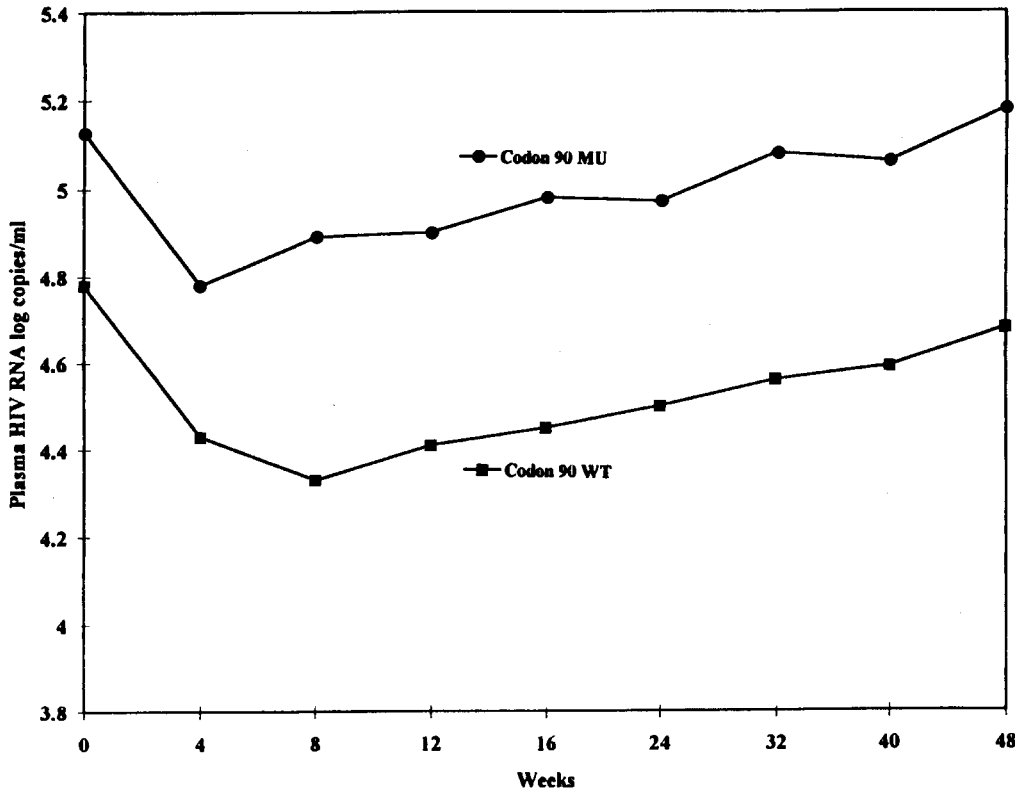

Figure 2. Changes in plasma human immunodeficiency virus (HIV) RNA levels in patients who developed codon 90 mutation (MU) to saquinavir in their HIV protease gene following 24–40 weeks of therapy and in those who retained wild type (WT) at codon 90.

velopment of the codon 90 mutation showed a trend toward statistical significance for the two-drug (33/72, 45.8%) versus the three-drug (24/72, 33.3%) regimen ($P = .11$, two-sided χ^2). The total number of mutations to saquinavir (codons 48 and 90) also showed a trend toward statistical significance for the two-drug (39/72, 54.2%) than for the three-drug (28/72, 38.9%) regimen ($P = .10$, two-sided χ^2).

Samples from 112 patients in both groups were also analyzed for mutations in the reverse transcriptase gene at codon 215, both before and after therapy. Mutations at codon 215 were present in 82% (92 patients) at baseline and in 87% (97) after therapy. There was no statistical difference in the proportion with codon 215 mutations before or after therapy between the zidovudine plus saquinavir and the zidovudine and zalcitabine plus saquinavir groups.

As was seen in the parent study, the changes in plasma HIV virus load for the group receiving zidovudine plus saquinavir and that receiving zidovudine and zalcitabine plus saquinavir showed a greater reduction for the three- versus two-drug combination (figure 1). There was no statistical difference in the baseline plasma HIV RNA values between the treatment groups.

Changes in plasma HIV RNA were plotted for patients who developed the codon 90 mutation following therapy and for those who did not (figure 2). The group in which the codon 90 mutation developed had a higher mean baseline plasma HIV RNA level (5.13 log RNA copies/mL, SD 0.58) than the group in which the mutation did not develop (4.79 log RNA copies/mL, SD 0.66) ($P < .01$). The maximal reduction in plasma HIV RNA in the patients developing the mutation was seen at week 4 and was 0.36 log RNA copies/mL. In the group not developing the codon 90 mutation, the maximal reduction was at week 8 and was 0.45 log RNA copies/mL. The plasma HIV RNA levels remained consistently lower in the group that did not develop the 90 mutation through week 48, although there was no statistical difference in the magnitude of the reduction from baseline between the 2 groups

The effect of the baseline reverse transcriptase codon 215 mutational status on the subsequent development of the protease codon 90 mutation was examined. There was no statistical difference in the rate at which mutations at codon 90 developed in patients with or without the codon 215 mutation at baseline.

Discussion

We studied the rate of development of key resistance mutations to saquinavir and zidovudine in highly zidovudine-

experienced patients receiving either a two-drug combination (zidovudine plus saquinavir) or a triple-drug combination (zidovudine and zalcitabine plus saquinavir). Although the difference did not reach statistical significance ($P = .11$), there was a trend toward a higher rate of development of mutations to saquinavir in patients receiving the two- versus the three-drug combination.

The number of mutations developing to saquinavir in this study was relatively high, considering that the great majority of patients were assayed after 24 weeks of therapy. In a review of early saquinavir-containing studies, Jacobsen et al. [14] found that 33% of patients on monotherapy had developed mutations by 4–6 months and 12.5%–63% after 7–12 months. Of interest, in a small study of overlapping patients from ACTG 229, Jacobsen et al. [14] reported that 12% and 22% of patients in the triple-drug combination group had mutations after 4–6 and 7–12 months, respectively. The differences between our findings and those previously reported may be due to the small number of patients previously studied (25 at 4–6 months and 18 at 7–12 months) and the specimens assayed (peripheral blood mononuclear cells vs. plasma) in the previous study. The high rate of saquinavir mutations in our study also may be related to the very high rate of baseline codon 215 mutations to zidovudine. Since most of the patients were already resistant to zidovudine at the start of therapy, little or no reduction in virus load was likely produced by the zidovudine component of the regimen, allowing mutations to saquinavir to develop at a higher rate.

The two-drug combination ($n = 72$) resulted in a total of 33 (46%) patients developing the codon 90 mutation compared with 24 (33%) of 72 receiving three drugs ($n = 72$). This trend may not have reached statistical significance due to the high rate of codon 90 mutation in both groups. This may be due to the high baseline virus load, to suboptimal exposure to saquinavir achieved with the standard dose used in this study, and to the high rate of baseline codon 215 mutations. Therefore, many patients in the triple-drug combination arm were essentially receiving active antiretrovirals in the form of zalcitabine and low-exposure saquinavir. This, coupled with the high virus load, would produce partial selective pressure on a highly replicating virus, a situation believed to result in high mutational rates [12, 15]. More potent antiretroviral regimens with greater drug exposure in the setting of less baseline drug resistance would be more likely to ultimately show benefit for three- versus two-drug regimens in reducing the development of mutations [4, 7].

The higher baseline and nadir virus load found in patients who developed the codon 90 mutation compared with those who did not supports the concept that higher viral replication increases the potential for resistance mutations to develop [15]. Recently, Kempf et al. [16] showed a strong correlation between the nadir of HIV RNA obtained after initiation of antiretroviral therapy and the duration of virus load suppression [16]. Lower nadirs resulted in a more durable response. Our results showing that lower nadirs result in a lower mutational rate would support their findings, as the lower mutational rate links the lower nadir to a more durable response.

In summary, we believe the results of this comprehensive mutational analysis of patients receiving dual versus triple combination therapy help substantiate our understanding of the mechanisms leading to drug resistance and support the current guidelines of administering triple combinations to obtain maximal viral suppression [1, 2].

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